The sample is combined with the reciprocal binding member, which may be bound to the support or subsequently bound to the support. After washing away the other components of the mixture, receptor for the target protein labeled with eTag reporter molecules specific for the particular receptor are added to the bound target protein, so as to become bound to the support through the target protein. One or more eTag reporter molecules will be bound to the receptor, usually not more than about 20, frequently not more than about 10. The number will be limited by the degree of loss of the binding affinity as the number of eTag reporter molecules is increased. Normally, the support bound receptor and the eTag reporter labeled receptor will bind to different epitopes of the target protein, although in some situations where the target has a plurality of the same epitope, the receptors may be specific for the same epitope. After washing away all eTag reporter labeled receptor that is not specifically bound to the target protein(s), the eTag reporter molecules are released and assayed.

Where the target permits binding of two reciprocal binding members or where an additional reagent is provided which permits this event, one can use determinations involving "channeling" or energy transfer. See, for example, U.S. Patent nos. 5,843,666 and 5,573,906. There are numerous methodologies involving channeling in the literature, where for the most part, the channeling was involved in producing a directly detectable signal, usually a change in absorption or emission of light. Channeling involves having two reagents, where the first reagent, when in proximity to the second reagent, produces a detectable signal. For the eTag reporter, the detectable signal is the release of the eTag reporter from the binding component. The release will usually be a function of the production of a short-lived entity, such as a chemical species or a photoactivated excited species, but may be the result of changing the local environment as compared to the bulk solution. So far as the chemical species, illustrative species include singlet oxygen, hydrogen peroxide, NADH, and hydroxyl radicals. Two entities are employed that have reciprocal binding members that bind to the same target moiety. One of the entities generates an active species. The other entity has a susceptible functionality that interacts with the active species resulting in release of the eTag reporter or responds to the changed local environment to release the eTag reporter. Either the active species is short lived, so that it will not create significant background because beyond its vicinity, the active species